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RESEARCH PAPER

In Vitro–In Vivo Correlation and Comparative Bioavailablity of Vincamine in Prolonged-Release Preparations

L. H. Emara,[1,*] B. S. El-Menshawi,[2] and M. Y. Estefan[3]

[1]Industrial Pharmacy Laboratory, Department of Pharmaceutical Sciences, [2]Department of Pharmacognosy, [3]Department of Medical Research, National Research Centre, El-Tahrir Str., Dokki, Giza 12622, Egypt

ABSTRACT

Developing an in vitro dissolution test that gives good correlation with in vivo data for a particular drug product is an important objective. Available dissolution data of vincamine prolonged-release preparations show different in vitro release behavior at different pH using the conventional dissolution techniques. This does not allow development of an in vitro–in vivo correlation (IVIVC). In the present work, the flow-through cell (FTC) dissolution system (USP apparatus 4) was utilized to compare the release rate of three marketed prolonged-release oral formulations of vincamine; namely, a brand innovator formulation used as the reference and two formulations from different manufacturers as test products. Both the open and closed systems of FTC were used at variable pH. A comparative bioavailability study was then conducted in 16 healthy volunteers for a test versus the reference product by administering a single dose of 60 mg in a crossover design. Vincamine plasma concentrations were analyzed by a sensitive high-performance liquid chromatography (HPLC) method. This was followed by assessment of IVIVC according to level A as specified by USP 23; the absorbed fraction of vincamine was determined using the Wagner-Nelson method. The results indicated that the pH of the medium affects the release rate pronouncedly. The relative bioavailability based on C_{max} *and* AUC_{0-12} *were found to be 83.15% and 84.15%, respectively. Good correlation was obtained between fraction absorbed in vivo and fraction dissolved in vitro by applying the open system of the FTC. This technique gave the most favorable results with regard to the percentage vincamine released and the IVIVC.*

Key Words: *Bioavailability; Dissolution; In vitro–in vivo correlation; Vincamine.*

* To whom correspondence should be addressed. Fax: +20-2-336-9603. E-mail: la_emara@hotmail.com

INTRODUCTION

To assess in vitro–in vivo correlations (IVIVC) for immediate- and extended-release oral dosage forms, comparisons of in vitro dissolution curves to the in vivo absorption curves are typically made. The in vitro dissolution curve usually is determined by a suitable dissolution test, and the in vivo absorption curve frequently is determined by deconvolution using model-dependent (e.g., Wagner-Nelson or Loo-Riegelman) (1) or direct mathematical deconvolution (2). Developing an in vitro dissolution test that gives a 1:1 IVIVC for a particular drug product is an important objective to facilitate product development and serves as a quality control procedure during product manufacture. Drug manufacturers typically use such tests to assess lot-to-lot variability and product shelf life and to predict in vivo performance (i.e., bioavailability) with reasonable assurance after conducting minor formulation and process changes (3). In the absence of a suitable in vitro test that can be interpolated to changes in drug plasma concentration-time profiles, appropriate testing in humans may have to be carried out, which can add much to the development costs of pharmaceutical formulations (4).

Potential problems in correlations of bioavailability and dissolution data originate from the complexity of drug absorption or the weakness of the dissolution design. Given that controlled-release dosage forms are now an increasingly important segment of newly introduced products, various regulatory bodies (e.g., FDA) are considering requiring the pharmaceutical industry to develop correlations for their products that can be used to support regulatory decisions (5,6).

There are obvious and insurmountable limitations to the official dissolution testing apparatus; for the rotating basket and paddle methods, this concerns the maintenance of sink conditions for drugs that saturate rapidly in a large volume of medium (7). For slightly soluble drugs, the limited volume of 1000 ml becomes critical with regard to the sink requirements; hence, larger dissolution containers have been proposed. However, these are complicated procedures (7).

The model character of the test requires, at least in special cases, a change of pH. This presents problems with all so-called basket methods. The inhomogeneity of the solution in the rotating basket and poor reproducibility led to enhanced use of the paddle apparatus for testing tablets and capsules. With the paddle apparatus, ''sinkers'' are necessary to avoid initial floating, but no agreement on a suitable design could be achieved, and poor reproducibility still exists (7).

The flow-through cell (FTC) model of a dissolution system effectively solves the problem of nonsink conditions by supplying an unlimited quantity of fresh dissolution medium with a convenient change of pH. The FTC has been incorporated in the DAC 1984 (7,8) and has recently been accepted officially as USP apparatus 4 (9), but its use is still not very widespread. This method offers distinct advantages in comparison with the USP paddle-and-basket method, especially for drugs with poor solubility and wettability.

In the present work, and based on the above considerations, three prolonged-release products (PRPs) of vincamine were studied in vitro utilizing the FTC apparatus 4 to establish a dissolution requirement for a PRP, followed by a comparative bioavailability study in humans of two products and assessment of the IVIVC.

Vincamine is known to be solubility/dissolution pH dependent (10), and its PRPs were found to possess varying in vitro dissolution characteristics. A few studies have attempted to correlate the in vitro dissolution with in vivo absorption of oral vincamine formulations (11–14), and they came to the conclusion that the in vitro data could not predict the actual in vivo behavior. Vincamine is an alkaloid derived from the small periwinkle (*Vinca minor*) and has a selective vasoregulating action on cerebral circulation. Its short half-life and the long-term treatment associated with its use justify the development of oral prolonged-release dosage forms (10).

EXPERIMENTAL

Studied Formulations

Three marketed prolonged-release oral formulations of vincamine in the form of coated pellets (30 mg per capsule) were studied and were designated as product I (Vincapront®, Mack, Germany), a brand innovator formulation used as a reference, and product II (Depotvinc®, October Pharma, Egypt) and product III (Oxybral®, Amoun, Egypt) as test products.

Materials

The following chemicals were analytical grade and the solvents used for high-performance liquid chromatography (HPLC) analysis were chromatographic grade: vincamine, ethylene diamine tetraacetic acid (EDTA), boric acid (Sigma, St. Louis, MO); methanol, acetonitrile, triethylamine, orthophosphoric acid (Merck, Germany); potassium dihydrogenphosphate (BDH, England); and sodium hydroxide (Prolabo, France).

Comparative In Vitro Release Studies Using Flow Through Cell

The comparative in vitro release studies using the FTC were carried out with a small FTC (Dissotest ® CE-6, connected with a piston pump Y 7; Sotax AG, Basel, Switzerland), representing the USP apparatus 4 (9), using both the closed (recirculating) and open systems. The flow rate (laminar) of the dissolution medium was 8.0 (±0.2) ml/min adjusted at 37°C. The dissolution media were simulated gastric fluid without enzyme (pH 1.2 and 4.5) and simulated intestinal fluid without enzyme (pH 6.9 and 7.5). The solutions were filtered (0.45 μm) and degassed by the solvent clarification kit (Millipore, USA). Fractions were collected at specified time intervals for quantitative analysis of vincamine, released from the test products, by ultraviolet (UV) measurement at 267 nm (Beckman DU 650 Spectrophotometer, USA). Each of the three products was tested in six cells simultaneously, and the means were calculated.

Flow-Through Cell Closed System

The four dissolution media, 900 ml each, were employed separately. Samples of 10 ml were removed periodically, replaced by the same quantity of the release medium and subsequently analyzed for vincamine levels.

Flow-Through Cell Open System

The dissolution media were allowed to circulate successively according to the scheme 1 hr (pH 1.2), 1 hr (pH 4.5), 2 hr (pH 6.9), and finally 1 hr (pH 7.5).

Comparative Bioavailability Study

Subjects and Protocol

Products I and II were subjected to single-dose comparative pharmacokinetic studies. The study protocol was approved ethically by the Committee of the Medical Unit of the National Research Center—Egypt. The healthy volunteers were selected, and the study was conducted according to internationally accepted guidelines and recommendations (9,15) and in the spirit of the revised Helsinki Declaration (Hong Kong, 1989) (16). The study design was a single-dose, fasting, two-treatment, two-period, two-sequence crossover comparing equal doses of the test and reference products; there was a 2-week washout period between the two phases of the study. An equal number of subjects was assigned randomly to the two dosing sequences. The16 healthy male volunteers between the ages of 21 and 48 years, assessed as healthy based on medical history, physical, and routine laboratory examinations, were admitted to the study treatment. All subjects gave written informed consent before entering the study.

Treatment and Sample Collection

Two capsules (equivalent to 60 mg) of product I or II were administered as a single dose to the overnight-fasting (minimum 10-hr fast) subject. Volunteers were instructed not to intake alcohol or xanthine-containing foods or beverages (coffee, tea, etc.) and nicotine for 48 hr prior to dosing and until after the last blood sample was collected. Venous blood samples were taken by direct venipuncture at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 12 hr. A 24-hr sample was taken from only 6 volunteers. The samples were placed in evacuated blood collection tubes and spiked with a trace of EDTA. Following centrifugation, the plasma samples were immediately frozen and stored (−18°C to −20°C) until assayed.

Sample Processing

Each of the test or standard plasma samples was subjected to an extraction procedure as follows (17). Frozen samples were allowed to thaw at room temperature. To 1 ml of plasma, 0.5 ml of 0.5 M borate buffer (pH 9) and 5 ml of methyl *tert*-butyl ether were added to 12-ml stoppered tubes. After vortex mixing (1 min) and centrifuging (3 min at 1500*g*), the tubes were placed in a deep-freezer (10 min at −80°C). The ether phase was transferred to another series of stoppered test tubes containing 150 μl of 0.017 M H_3PO_4 and back-extracted by agitating for 1 min on a vortex mixer. After centrifuging for 3 min at 1500*g*, a 100-μl aliquot of the lower acidic phase was injected onto the HPLC column.

Standard Solutions and Calibration Standards

A stock solution of vincamine was prepared in methanol at a concentration of 10 mg/50 ml. This stock solution was then diluted further to yield appropriate working solutions for the preparation of the calibration standards. The calibration standards were prepared by adding 20 μl of a suitable standard solution to drug-free plasma to obtain vincamine concentrations in the range 2.5–300 ng/ml. The spiked plasma samples were then treated as described in the next section. A linear relation was established between vincamine standard concentrations and the peak height. Linear least-square regression line of the constructed standard curve was computed using the Base-

line 810 computer program (Waters Assoc., Milford, MA). The percentage recoveries were 87%, 112.3%, 90%, and 101 %, for 2.5, 20, 40, and 300 ng/ml, respectively. The correlation coefficient was found to be 0.99926.

Bioanalytical Method

For the determination of vincamine in human plasma samples, the reported HPLC-UV method of Bo et al. (17) was followed in principle. The developed method was examined and validated.

High-Performance Liquid Chromatography Apparatus and Chromatographic Parameters

Analyses were carried out using a Waters 600E Multisolvent Delivery System high-performance liquid chromatograph equipped with a model U6K injector and 484 Waters tunable absorbance detector set at 232 nm, coupled to Baseline 810 computer program. The analytical column used was a Prodigy ODS 5-μ 100 A column, 250 mm × 4.6 mm i.d., 5-μm particle size (Phenomenex, USA) protected by a guard pack precolumn module with Bondapak C18 inserts (Waters Assoc.). The column temperature was controlled at 50°C by a temperature control module (TCM, Waters Assoc.).

The mobile phase consisted of acetonitrile–0.02 M potassium dihydrogenphosphate containing 0.1% triethylamine with the pH adjusted to 3 with 1 M phosphoric acid (30:70, v/v) and filtered through a 0.45-μm membrane (Millipore). The chromatography was performed isocratically at a flow rate of 1.5 ml/min. The chromatographic process was controlled by the Baseline 810 computer program.

Pharmacokinetic Characteristics

The individual pharmacokinetic parameters of vincamine were derived by noncompartmental analysis (18) using the WinNonLin-Pro 2.1 computer program (Pharsight, NC). The following parameters were derived: the peak plasma concentration C_{max} and the time to reach peak plasma concentration t_{max} (both were observed values); the apparent elimination half-life of vincamine $t_{1/2}$ as determined by log-linear regression analysis of the terminal portion of the plasma concentration-time curve of vincamine; $AUC_{0\text{-}12}$ and $AUC_{0\text{-}\infty}$ by the linear trapezoidal rule and extrapolation to infinity, respectively. Mean residence time (MRT) of the drug in the systemic circulation was calculated by MRT = AUMC/AUC.

In Vivo–In Vitro Correlation

To calculate the IVIVC, the rate of vincamine absorption in the human test subjects was determined from the plasma vincamine concentration-time data and calculated by the Wagner-Nelson method (1). Linear regression analysis was applied between the plots of the fraction of drug dissolved in vitro and the fraction of drug absorbed in vivo (9).

RESULTS AND DISCUSSION

Comparative In Vitro Release Studies Using Flow Through Cell

Closed System

Dissolution of each product in a fixed volume under various pH gave release rates of vincamine as recorded in Figs. 1A–1D. The pH variation resulted in substantial differences in the dissolution profiles of the products tested, a fact that calls for some comments. In acidic pH 1.2 (Fig. 1A), product III was characterized by a relatively rapid release rate (35.16%) during the first hour compared to 14.59% and 15.9% for products I and II, respectively. After 5 hr, the percentage vincamine released reached 50.99%, 31.94%, and 56.57%, for products I, II, and III, respectively. However, product III possessed an irregular release rate in pH 1.2 (Fig. 1A). While products I and II showed coinciding release rates during the first hour, the release rate of product I was markedly increased after this hour compared to product II (Fig. 1A). On increasing the pH of the dissolution medium to 4.5 (Fig. 1B), the percentage vincamine released was markedly decreased. Product III showed the minimum amount of vincamine released (6.66%) after 5 hr, while products I and II released 13.55% and 17.16% vincamine, respectively. Further increase in pH to 6.9 (Fig. 1C), resulted in an increase of percentage vincamine released from products I and II, while product III showed the minimum value. At pH 7.5 (Fig. 1D), product I gave the higher amount of vincamine released (41.5% after 5 hr), and on the other hand, the amount decreased from products I and III (24.55% and 18.55%, respectively, Fig. 1D).

These data indicate that vincamine release rate from the three products was highly influenced by the pH of the media. This means that conditions of absorption will be much dependent on such physiological parameters as stomach-emptying time, rate of intestinal transit, and variations in pH. Such data could not be used as criteria for prediction of its bioavailability. Furthermore, the con-

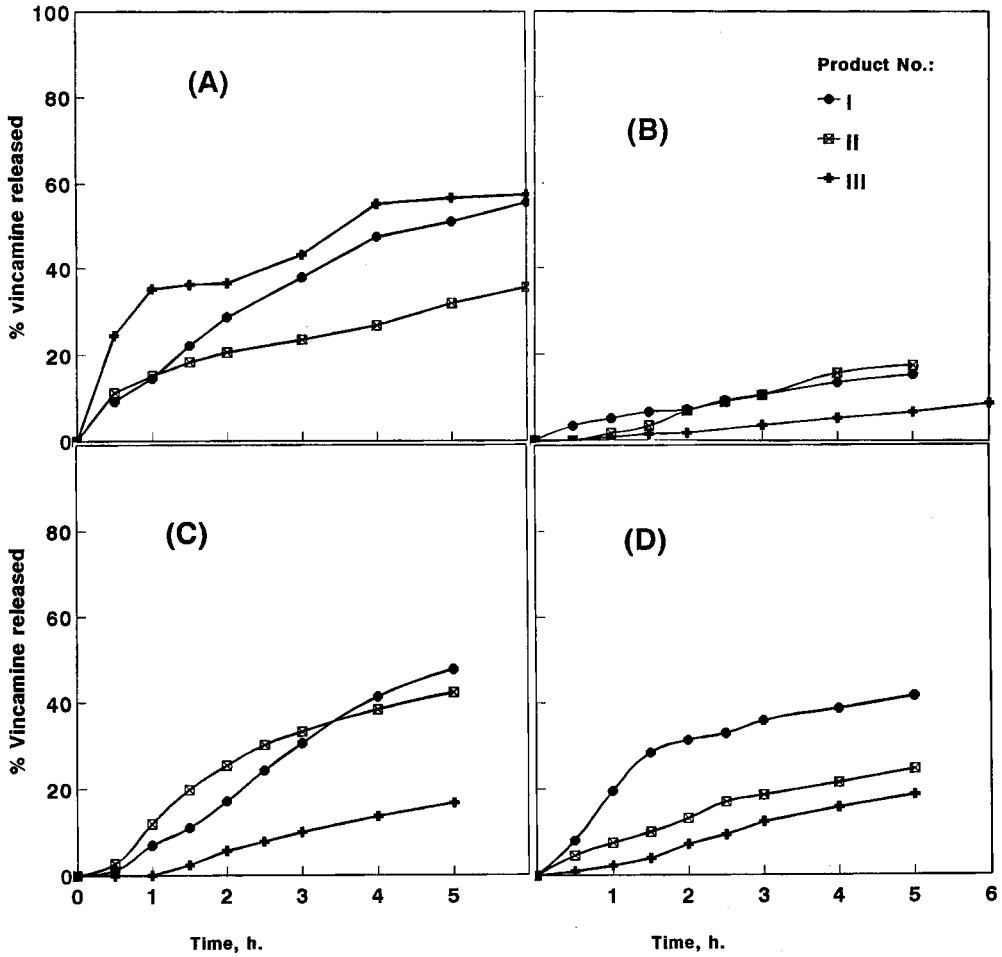

Figure 1. Release patterns of 30-mg vincamine PRP using the closed system of the flow-through cell: (A) pH 1.2; (B) pH 4.5; (C) pH 6.9; and (D) pH 7.5.

centration buildup in the case of dissolution studies carried out at fixed volume of dissolution medium is different from the in vivo process, in which the dissolved drug is removed continuously from the liquid by absorption (7). Buri et al. (10) obtained similar results from six commercial vincamine PRPs at various pH using round-bottom flasks contained 1000 ml of dissolution medium. The low solubility of vincamine in media with pH near to neutral is one of the principal reasons for the variability in the release rates (19).

Open System

The open system was used to ensure perfect sink conditions and regular change of pH, covering the range 1.2 to 7.5. Under these conditions, the results clarified a biphasic release rate (Fig. 2). In the case of products I and II, a slow phase was obtained during the first hour, giving almost the same amount of vincamine released (15.6% and 15.9%, respectively), and this was followed by a rapid phase, in which a total of 93.58% and 84.74% were released after 5 hr, respectively. Contrarily, product III showed first a rapid phase that prolonged up to 2 hr, giving 38.59% and 65% release after 1 and 2 hr, respectively, which was followed by a slower phase during which a total of 85% vincamine was released after 5 hr. Under the previous experimental conditions, the open system of FTC with gradient pH variation might represent a favorable technique for evaluating a PRP. This system provides a progressive dissolution that may figure out the continuous absorption during passage at different pH values through the whole gastrointestinal tract. It is

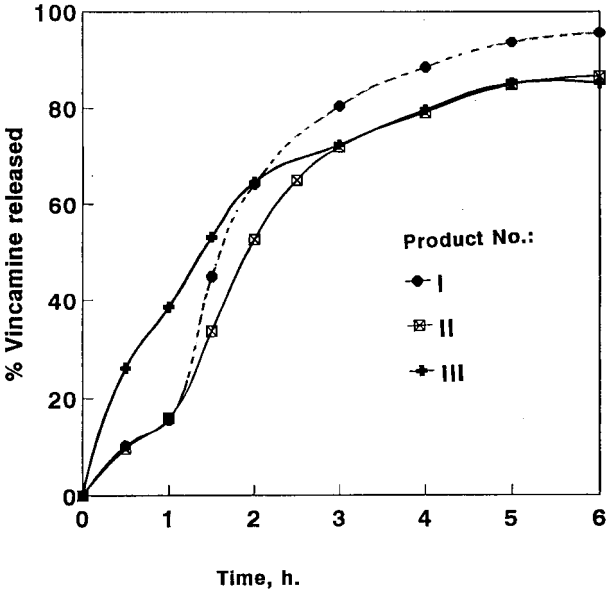

Figure 2. Release patterns of 30-mg vincamine PRP using the open system of the flow-through cell at gradient changes of pH.

assumed that only a comparable in vivo study would provide confirmation of this hypothesis.

Comparative Bioavailability Study

Products I and II were selected for this study as they possess almost the same in vitro release profiles in the FTC open system (Fig. 2). The mean vincamine plasma concentration-versus-time profiles for the two treatments are shown in Fig. 3. The results of pharmacokinetic parameters appear in Table 1. Following administration of product I, the mean maximum plasma vincamine concentration C_{max} was 91.67 ng/ml (range 19.43–137.13 ng/ml). Following product II administration, the values were significantly lower ($p \leq .05$) with a mean C_{max} of 76.22 ng/ml (range 12.56–167.49 ng/ml). The relative bioavailability based on C_{max} was found to be 83.15%. There was no significant difference in time to maximum concentration t_{max}, although there was a trend toward later values following product I treatment. The overall bioavailability judged from the $AUC_{0\text{-}12}$ was found to be 84.15%.

On the basis of the MRT value, which is a characteristic parameter in the retarding nature of preparations, the retarding characters of the two products were identified.

These data are almost comparable to the report of Steinbach et al. (14) on Vincapront retard, which possessed a C_{max} of 103 ng/ml, t_{max} of 1.8 hr, $t_{1/2}$ of 4.3 hr, and an AUC of 507 ng $\cdot$ ml^{-1} hr.

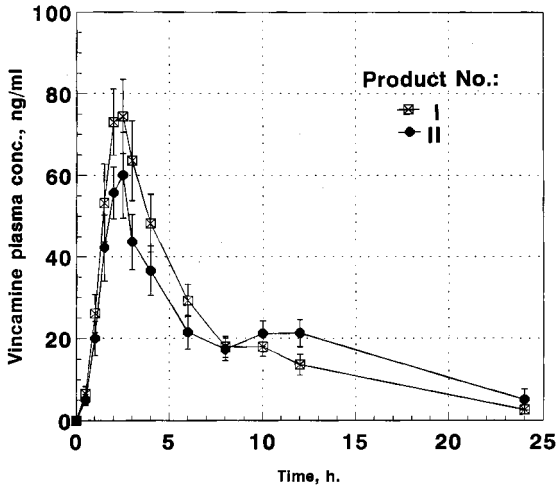

Figure 3. Mean vincamine plasma concentrations as a function of time for the two treatments ($n = 16 \pm$ SE).

A small absorption peak, markedly lower than the first one, was detectable in most of the volunteers 10 hr after administration (Fig. 3) and could be attributed to the late absorption of vincamine in the upper and lower jejunum. In only 11 volunteers, during the two treatments, another peak was also detected 3 to 4 hr after administration (Fig. 4), which might be due to enterohepatic cycling. This result agrees with the data reported for oral vincamine adenhydrate aqueous solution by Aiache et al. (20), who also found a lower peak, in some subjects, detectable 2 to 3 hr after administration.

Table 1 shows a marked prolongation in the $t_{1/2}$ for product II, with a mean of 7.50 hr (±SE 3.97), while in product I, the mean $t_{1/2}$ value was 4.18 hr (±SE 0.86).

In Vitro–In Vivo Correlation

The USP 23 (9) defines IVIVC as the establishment of a relation between a biological property, or one or more

Table 1

In Vivo Data for Vincamine Sustained-Release Products

Parameter	Product I	Product II
Dose (mg)	60	60
C_{max} ($ng.ml^{-1}$)	91.67 (±10.26)	76.22 (±10.10)
T_{max} (hr)	2.38 (±0.17)	2.13 (±0.17)
$AUC_{0\text{-}12}$ ($ng.ml^{-1}hr$)	380.40 (±40.60)	320.12 (±39.47)
$AUC_{0\text{-}\infty}$ ($ng.ml^{-1}hr$)	514.65 (±70.42)	693.40 (±160.40)
MRT (hr)	4.72 (±0.10)	5.29 (±0.10)
$t_{1/2}$ (hr)	4.18 (±0.86)	7.50 (±3.97)

Each value is a mean ± SE.

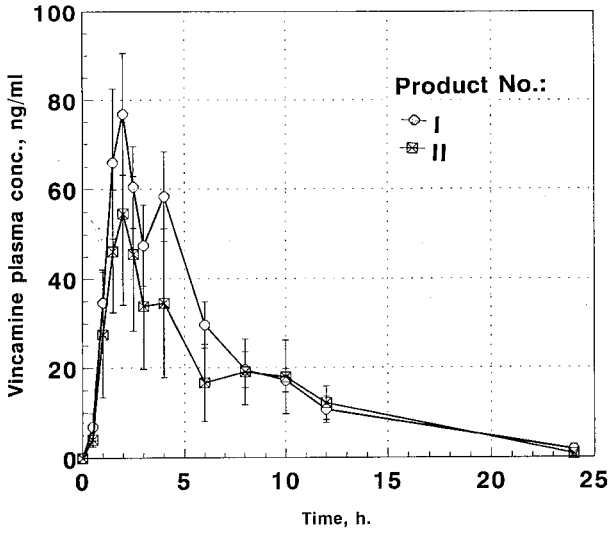

Figure 4. Mean vincamine plasma concentrations as a function of time for the two treatments for selected subjects.

pharmacokinetic parameters of a dosage form, and the physiochemical characteristics (e.g., in vitro dissolution mechanism) of the same dosage form. USP 23 also introduced a classification system of correlation methods for extended-release dosage forms that specifies three levels (designated A, B, and C), listed in the order of descending quality. The preferred correlation for controlled-release delivery systems is level A, (9,21), for which the in vitro dissolution profile is compared to the in vivo dissolution curve and should be essentially superimposable. Level A correlations are favorable first because each plasma concentration and dissolution time point generated is used and thus reflects the whole curve; second, they are excellent quality control procedures since in vivo performance of a particular dosage form can be predicted by an in vitro test; third, the boundaries of the in vitro dissolution curve can be justified on the basis of convolution or deconvolution (1,2).

In this study, a linear relation was obtained between the fraction absorbed in vivo and the fraction dissolved in vitro according to different dissolution techniques (Figs. 5 and 6). In case of the FTC open system, Figures

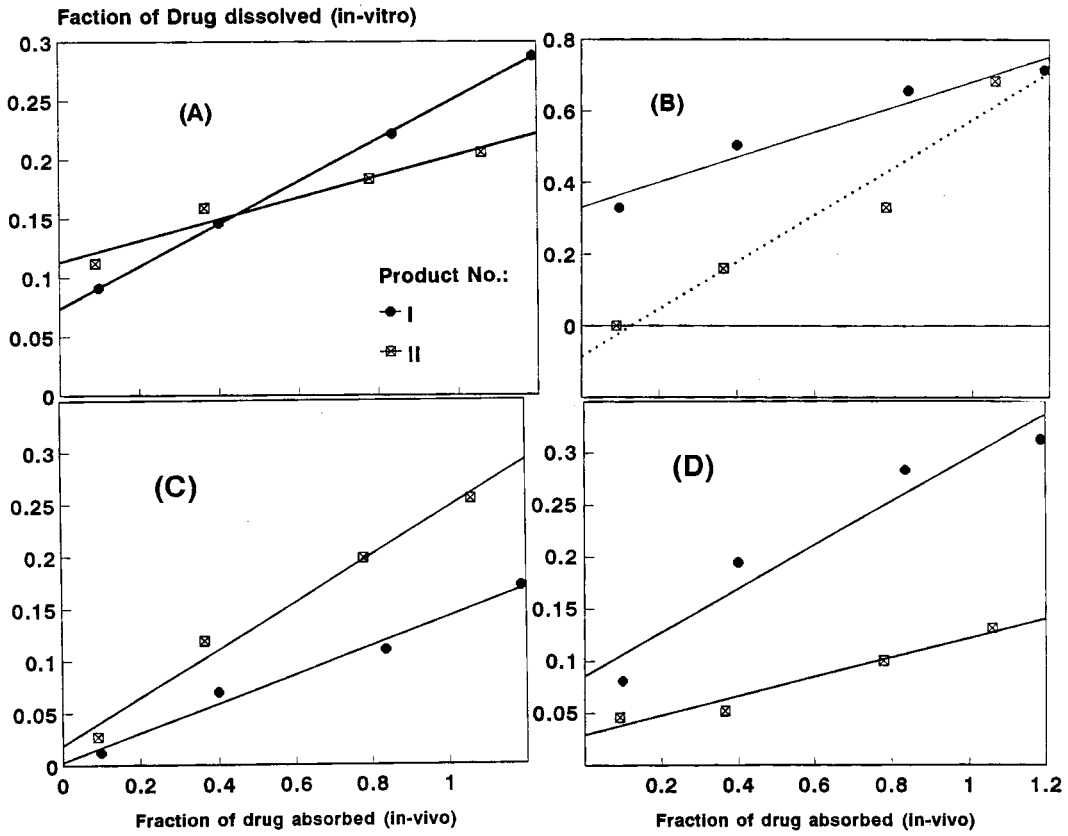

Figure 5. IVIVC of the fraction absorbed calculated from plasma and fraction dissolved in vitro using the closed system of the flow-through cell: (A) pH 1.2; (B) pH 4.5; (C) pH 6.9; and (D) pH 7.5.

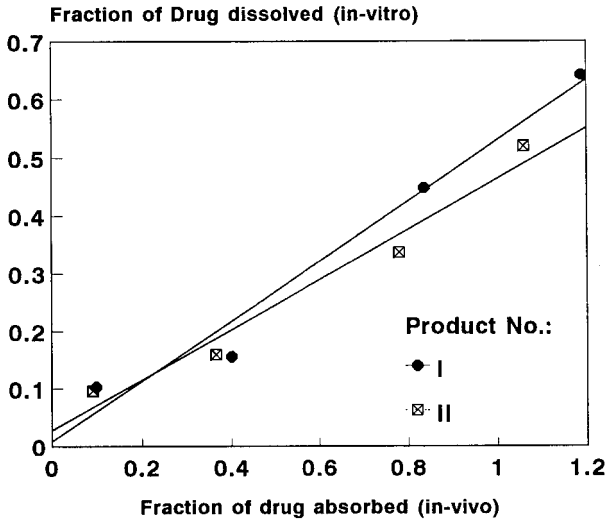

Figure 6. IVIVC of the fraction absorbed calculated from plasma and fraction dissolved in vitro using the open system of the flow-through cell at gradient changes of pH.

2 and 7 have similar appearances, and this is translated to similar IVIVC (Fig. 6), displaying good linearity ($r = .98$). Figure 6 shows a y intercept that is reasonably close to zero (Table 2). The computed slopes (Table 2) showed values that were deviated from 1.0; however, the slopes of the two regression lines displayed in Fig. 6 showed the highest values (Table 2). Contrarily, the FTC

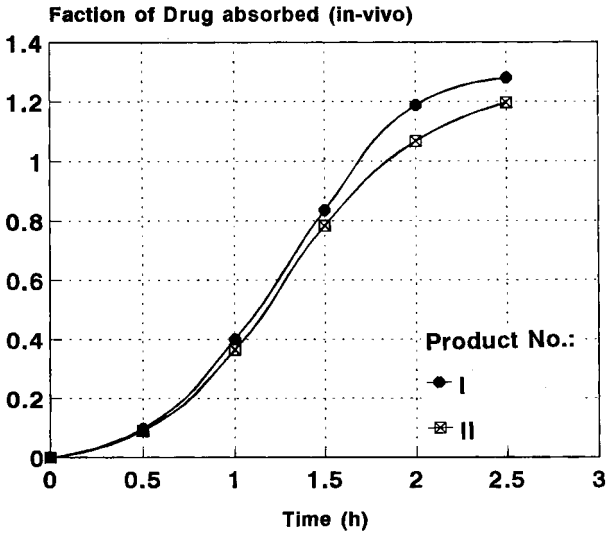

Figure 7. Fraction of vincamine absorbed-time profiles in vivo for the two products administered to 16 human subjects.

Table 2

Regression Results for In Vitro–In Vivo Correlation

Flow-Through System	pH		Linear Regression Data	
			Product I	Product II
Open	Variable	*r*	.985	.9840
		Slope	0.520	0.4400
		Intercept	0.009	0.0280
Closed	1.2	*r*	.9999	.9730
		Slope	0.1800	0.0908
		Intercept	0.0740	0.1130
Closed	4.5	*r*	.974	.9700
		Slope	0.350	0.6600
		Intercept	0.330	−0.0860
Closed	6.9	*r*	.9918	.9910
		Slope	0.1400	0.2300
		Intercept	0.0190	0.0030
Closed	7.5	*r*	.9644	.9785
		Slope	0.2100	0.0900
		Intercept	0.0860	0.0300

closed system showed that the fraction of vincamine released when plotted against the fraction of vincamine absorbed gave less satisfactory results (Table 2 and Fig. 5).

There was no similarity observed between the fraction of vincamine absorbed (Fig. 7) with both the in vitro release curves (Fig. 1) and the IVIVC (Fig. 5). Also, the values of y intercept and slopes showed a larger deviation from zero and from unity, respectively (Table 2 and Fig. 5).

Skelly et al. (22) stated that a level A correlation is most applicable to controlled-release formulations that demonstrate in vitro drug release rates that are essentially independent of the dissolution medium. Giving that vincamine is considered solubility/dissolution pH dependent, as proved by the present study and others (10), a 1:1 IVIVC cannot be expected (23). This should be kept in mind when presenting a 1:1 IVIVC for certain drug products; moreover, the complete process of developing an IVIVC with high quality and predictability, and identifying specific applications for such correlations, has still not been well defined (24). However, the present study indicates that application of an open system with variable pH range of a flow-through dissolution test for vincamine PRP is one simple approach that can be used to obtain reasonable IVIVC and can potentially facilitate future improvements in the design of the product and benefit the development and testing of similar formulations of other drugs that are poorly water soluble drugs.

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